Primary Reviewer: Milutin Djurickovic, EPA/BPPD/MPB **Date:** SEP 2 5 2017

Secondary Reviewer: Shannon Borges, EPA/BPPD/MPB **Date:** SEP 2 5 2017

DATA EVALUATION RECORD

REQUIREMENT: U.S. EPA OCSPP Guideline: 885.4050–Avian Oral Toxicity
U.S. EPA OCSPP Guideline: 885.4100–Avian Inhalation Toxicity
U.S. EPA OCSPP Guideline: 885.4150–Wild Mammal Testing
U.S. EPA OCSPP Guideline: 885.4200–Freshwater Fish Testing
U.S. EPA OCSPP Guideline: 885.4240–Freshwater Invertebrate Testing
U.S. EPA OCSPP Guideline: 885.4280–Marine/Estuarine Animal Testing
U.S. EPA OCSPP Guideline: 885.4300–Nontarget Plant Testing
U.S. EPA OCSPP Guideline: 885.4340–Nontarget Insect Testing

TEST MATERIAL: *Clonostachys rosea* CR-7 Technical, Vectorite

CITATION: Beth Mileson (TSG, Inc.). 2016. Supplemental Response to Tier 1 Microbial Pesticide Data Requirements for *Clonostachys rosea* CR-7 Technical. Sponsored by Bee Vectoring Technology Inc., 4160 Sladeview Crescent #7, Mississauga, ON L5L 0A1, Canada. December 22, 2016. Unpublished MRID No. 50139701 (replaces MRID 49949210).

SPONSOR: Bee Vectoring Technology Inc., 4160 Sladeview Crescent #7, Mississauga, ON L5L 0A1, Canada

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. The study was not conducted in compliance with GLP [40 CFR § 160]. The study is not required to be GLP, since it is a waiver request. This DER does not contain FIFRA CBI.

CLASSIFICATION: ACCEPTABLE

I. OCSPP 885.4050 – Avian Oral Toxicity & OCSPP 885.4100 – Avian Inhalation Toxicity

A. **RATIONALE:** *C. rosea* is ubiquitous in the environment. It has been isolated from both aquatic and terrestrial plants. Examples of this include the following; sixteen strains of *C. rosea* were isolated from different types of ecosystems in Brazil, from plant tissues of coffee, pear, peach, rose, eucalyptus, and soil samples (Nobre et al., 2005). Five isolates were identified in barley, seed, straw, and leaves. While 25 isolates were found in fresh carrots, peels, and seeds in Denmark (Jensen et al., 2004). Strains were isolated from strawberry plants in Canada (McLean & Sutton, 1991), from an estuary in France, and another from seaweed in Micronesia (Dias et al., 2015; Tomoda et al., 1999).

C. rosea is a fungal endophyte that colonizes plants near the place of entry. The presence of *C. rosea* protects plants from diseases (Karlsson et al., 2015) and then sporulates when plants begin to senesce. *C. rosea* CR-7 vectored by bees is not expected to increase levels of the microbe above natural levels due to competition from other microorganisms (Peay et al., 2008) and exposure to ultraviolet radiation which decreases *C. rosea* populations (Costa et al., 2013).

In summary no hazard is expected for avian species due to the following; 1) birds are naturally exposed to *C. rosea* as a result of the ubiquitous distribution of this microbe in the environment, 2) the application method of *C. rosea* strain CR-7 by bee vectoring small quantities of end use product suggest that exposure to birds following application will be low, and 3) the microbial ecology of *C. rosea* and the dynamic microbial community indicate exposure to birds will not be increased appreciably throughout the life cycle of the microbe.

B. EPA REVIEW: The reviewer mostly agrees with the presented rationale, however additional points must be included for clarification. The use of *C. rosea* CR-7 will of course increase the amount of *C. rosea* present in agroecosystems on mostly flowers and developing fruit during the time of application, flower bloom, and after plant senescence where birds will be exposed to these increased levels. After senescence and harvest the amount of *C. rosea* CR-7 will decrease and return to natural background levels due to removal of fruit, competition from other microorganisms and degradation from ultraviolet radiation (Costa et al., 2013)/temperature changes/precipitation. Avian species are and have been exposed to *C. rosea* due to its ubiquity, but since the optimal growth temperature of *C. rosea* CR-7 is 20 to 28° C as reported in the product characterization data, it is not expected to grow in birds or be toxic/pathogenic to them.

II. OCSPP 885.4150 – Wild Mammal Testing

A. RATIONALE: An acute oral toxicity study conducted to evaluate the potential effects of a limit dose of CR-7 Technical (50% a.i.) on rats indicated the test material is low in acute toxicity following oral exposure (Hartwell, 2016) (MRID No. 499492-03). Female albino rats received a dose of 5,000 mg/kg CR-7 Technical by oral gavage and were monitored for effects for two weeks. No mortality occurred during the study and the rats exhibited no clinical signs of toxicity. All animals gained weight each week. The acute oral LD_{50} for CR-7 Technical was identified as > 5,000 mg/kg in female albino rats.

A Pulmonary Toxicity/Pathogenicity study conducted to evaluate the potential effects in rats of a single high dose pulmonary exposure to pure *C. rosea* strain CR-7 indicated the test material is not toxic or pathogenic following acute exposure to the respiratory system (Doig, 2016) (MRID No. 499492-02). Treated rats received by tracheal injection, a single dose of 0.3 ml *C. rosea* strain CR-7 with counts of 3.1×10^8 CFU /ml. Rats were observed often on the day of dosing and once daily thereafter for 21 days. Interim sacrifices of treated rats were conducted on days 0, 3, 7, 14, and 21, and blood and tissue samples were collected from these subjects at each sacrifice and cultured to quantify the clearance pattern of the microbe. There were no abnormal health observations and no abnormalities identified during necropsy. The test organism was not detected in any tissues at any time point, and was considered cleared from all tissues and blood

by day 21. The test substance, *C. rosea* strain CR-7, was determined to be non-toxic and non-pathogenic following tracheal injection exposure to a single dose of 3.1×10^8 CFU /ml.

In summary no hazard is expected for wild mammals due to the following reasons: 1) *C. rosea* strain CR-7 was not toxic or pathogenic to rats following acute exposures, 2) there is no reason to believe that tests required to assess human and domestic animal health hazards are inadequate or inappropriate for assessment of hazards to wild animals, and 3) the proposed use of the MPCA is not expected to increase the likely exposure to *C. rosea* above natural background levels.

B. EPA REVIEW: The reviewer mostly agrees with the presented rationale. The *C. rosea* strain CR-7 is not expected to be hazardous to wild mammals. Exposure could be high during application periods, but no hazard from exposure is expected.

III. OCSPP 885.4200, 885.4240, 885.4280 – Freshwater Fish Testing, Freshwater Invertebrate Testing, Marine/Estuarine Fish and Invertebrate Testing

A. RATIONALE: *C. rosea* is ubiquitous in the environment and fish and aquatic invertebrates are naturally exposed to the microbe. It has been isolated from both aquatic and terrestrial plants. Examples of this include the following; sixteen strains of *C. rosea* were isolated from different types of ecosystems in Brazil, from plant tissues of coffee, pear, peach, rose, eucalyptus, and soil samples (Nobre et al., 2005). Five isolates were identified in barley, seed, straw, and leaves. While 25 isolates were found in fresh carrots, peels, and seeds in Denmark (Jensen et al., 2004). Strains were isolated from strawberry plants in Canada (McLean & Sutton, 1991), from an estuary in France, and another from seaweed in Micronesia (Dias et al., 2015; Tomoda et al., 1999).

The application rate (27 g a.i./acre) on the flowers common to fruit and nut trees during bloom, is not expected to create significant exposure to aquatic systems above natural levels.

C. rosea is a fungal endophyte that colonizes plants near the place of entry. The presence of *C. rosea* protects plants from diseases (Karlsson et al., 2015) and then sporulating when plants begin to senesce. *C. rosea* CR-7 vectored by bees is not expected to increase levels of the microbe above natural levels due to competition from other microorganisms (Peay et al., 2008) and exposure to ultraviolet radiation which decreases *C. rosea* populations (Costa et al., 2013).

In summary no hazard is expected during exposure, and minimal exposure is expected based on the following points; 1) fish are naturally exposed to *C. rosea* as a result of the ubiquitous distribution of this microbe in the environment, 2) the application method of *C. rosea* strain CR-7 by bee vectoring small quantities of end use product suggest that exposure to fish following application will be low, and 3) the microbial ecology of *C. rosea* and the dynamic microbial community indicate exposure to fish will not be increased appreciably throughout the life cycle of the microbe.

B. EPA REVIEW: The reviewer agrees with the presented rationale, however additional points must be included for clarification. The use of *C. rosea* CR-7 will of course increase the amount of *C. rosea* present in agroecosystems on mostly flowers and developing fruit during the time of application, flower bloom, and after plant senescence. After senescence and harvest the amount

of *C. rosea* CR-7 will decrease and return to natural background levels due to removal of fruit, competition from other microorganisms and degradation from ultraviolet radiation (Costa et al., 2013)/temperature changes/precipitation. Overall, no hazard is expected from exposure to *C. rosea* CR-7 due to its ubiquity in the environment, and exposure will be minimal even during application periods due to low numbers of spores that will runoff into waterbodies.

IV. OCSPP 885.4300 – Nontarget Plant Testing

A. RATIONALE: *C. rosea* is ubiquitous in the environment. It has been isolated from both aquatic and terrestrial plants. Examples of this include the following; Sixteen strains of *C. rosea* were isolated from different types of ecosystems in Brazil, from plant tissues of coffee, pear, peach, rose, eucalyptus, and soil samples (Nobre et al., 2005). Five isolates were identified in barley, seed, straw, and leaves. While 25 isolates were found in fresh carrots, peels, and seeds in Denmark (Jensen et al., 2004). Strains were isolated from strawberry plants in Canada (McLean & Sutton, 1991), from an estuary in France, and another from seaweed in Micronesia (Dias et al., 2015; Tomoda et al., 1999).

C. rosea use on a several types of plants have been evaluated mostly as a protective agent against grey mold *Botrytis cinerea*. *C. rosea* has provided protection against grey mold in black spruce seedling, begonia, cyclamen, tomato, and geranium plants compared to untreated controls (Sutton et al., 1997). When plants are inoculated with *C. rosea* grey mold sporulation is minimal. Furthermore, a variety of *C. rosea* strains were tested for protection of carrot seeds, and most of the strains offered protection from the two seedborne pathogens that were used to challenge the *C. rosea* strains (Jensen et al., 2004). This research has also shown that all the *C. rosea* strains isolated from where Barely is grown offered protective benefits, while 15 of 25 *C. rosea* strains from carrot agroecosystems provided protective benefits.

Early reports on *C. rosea* discussed it's potential for pathogenicity to apple fruits, potato tubers, and other plants. That research is now considered inconclusive (Sutton et al., 1997). It is now understood that *C. rosea* does penetrate the plant host at an injured vulnerable point and colonizes the plant host without causing harm to the plant and only multiplies at senescence.

In summary, the following are the main points of the rationale; 1) all plants appear to be naturally exposed to *C. rosea* as a result of the ubiquitous distribution of this microbe in the environment with no adverse effects, 2) A variety of studies have demonstrated that *C. rosea* is a mycoparasitic fungus that confers beneficial effects on the plant host, not adverse effects, and 3) early reports of potential adverse effects of *C. rosea* on plants have been countered by more precise descriptions of the microbe interactions with plant hosts that indicate the effects were not adverse.

B. EPA REVIEW: The reviewer agrees with the presented rationale.

V. OCSPP 885.4340, 885.4380 – Nontarget Insect Testing

A. RATIONALE: *C. rosea* is ubiquitous in the environment and insects are naturally exposed to the microbe. It has been isolated from both aquatic and terrestrial environments. Examples of this include the following; sixteen strains of *C. rosea* were isolated from different types of

ecosystems in Brazil, from plant tissues of coffee, pear, peach, rose, eucalyptus, and soil samples (Nobre et al., 2005). Five isolates were identified in barley, seed, straw, and leaves. While 25 isolates were found in fresh carrots, peels, and seeds in Denmark (Jensen et al., 2004). A strain was isolated from strawberry plants in Canada (McLean & Sutton, 1991), from an estuary in France, and another from seaweed in Micronesia (Dias et al., 2015; Tomoda et al., 1999).

C. rosea is a fungal endophyte that colonizes plants near the place of entry. The presence of *C. rosea* protects plants from diseases (Karlsson et al., 2015) and then sporulating when plants begin to senesce. C. rosea CR-7 vectored by bees is not expected to increase levels of the microbe above natural levels due to competition from other microorganisms (Peay et al., 2008) and exposure to ultraviolet radiation which decreases *C. rosea* populations (Costa et al., 2013).

One report from the literature indicated that *C. rosea* is pathogenic to insects (Toledo et al., 2016, but this study is flawed. The test insects were collected in the field and stored for a period of time before they were inoculated with *C. rosea*. The insects were considered healthy, but no criteria were given for evaluating their health status. High mortalities were reported with low *C. rosea* sporulation. No evidence was provided that the limited *C. rosea* sporulation was actually the cause of mortality for the test insects.

The in-life portion of the honey bee study ended on day 16, according to protocol, when mortality in the control group exceeded 20%. There were no significant differences in the mean number of dead honey bees in the inactive and active groups on day 16 compared to the control group. Percent mortality in the control, inactive and active groups on day 16 was 21.3%, 26.7% and 20.7%, respectively. There were no significant differences in mortality among the groups. Bees in the active group on average, consumed significantly more food than bees in the control group. Based on the results of this study, the test substance, *C. rosea* Strain CR-7 Technical, in its inactive and active form was determined to be non-toxic to the honey bee after 16 days of being administered orally at 3.2×10^5 CFU/ml or 4.1×10^6 CFU/bee.

In summary the rationale consists of the following main points; 1) insects are naturally exposed to *Clonostachys rosea* as a result of the ubiquitous distribution of this microbe in the environment, 2) the application method of *C. rosea* strain CR-7 by bee vectoring small quantities of end use product suggest that exposure to insects following application will be low, 3) the microbial ecology of *C. rosea* and the dynamic microbial community indicate exposure to insects will not be increased appreciably throughout the life cycle of the microbe, 4) *C. rosea* strain CR-7 was not toxic to honey bees, and 5) information in the literature supports the position that this mycoparasite is not pathogenic to insects, and the reference identified to the contrary is scientifically limited.

B. EPA REVIEW: The reviewer agrees with the presented rationale, however additional points must be included for clarification. The use of *C. rosea* CR-7 will of course increase the amount of *C. rosea* present in agroecosystems on mostly flowers and developing fruit during the time of application, flower bloom, and after plant senescence which will expose insects to the higher levels present during application periods. After senescence and harvest the amount of *C. rosea* CR-7 will decrease and return to natural background levels due to removal of fruit, competition from other microorganisms and degradation from ultraviolet radiation (Costa et al.,

2013)/temperature changes/precipitation. Most importantly, *C. rosea* strain CR-7 was not toxic to honey bees therefore no hazard from exposure is expected.

VI. References

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